Development of Lewy Bodies Biofluid Signatures by Targeted Proteomics (U18)

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NINDS

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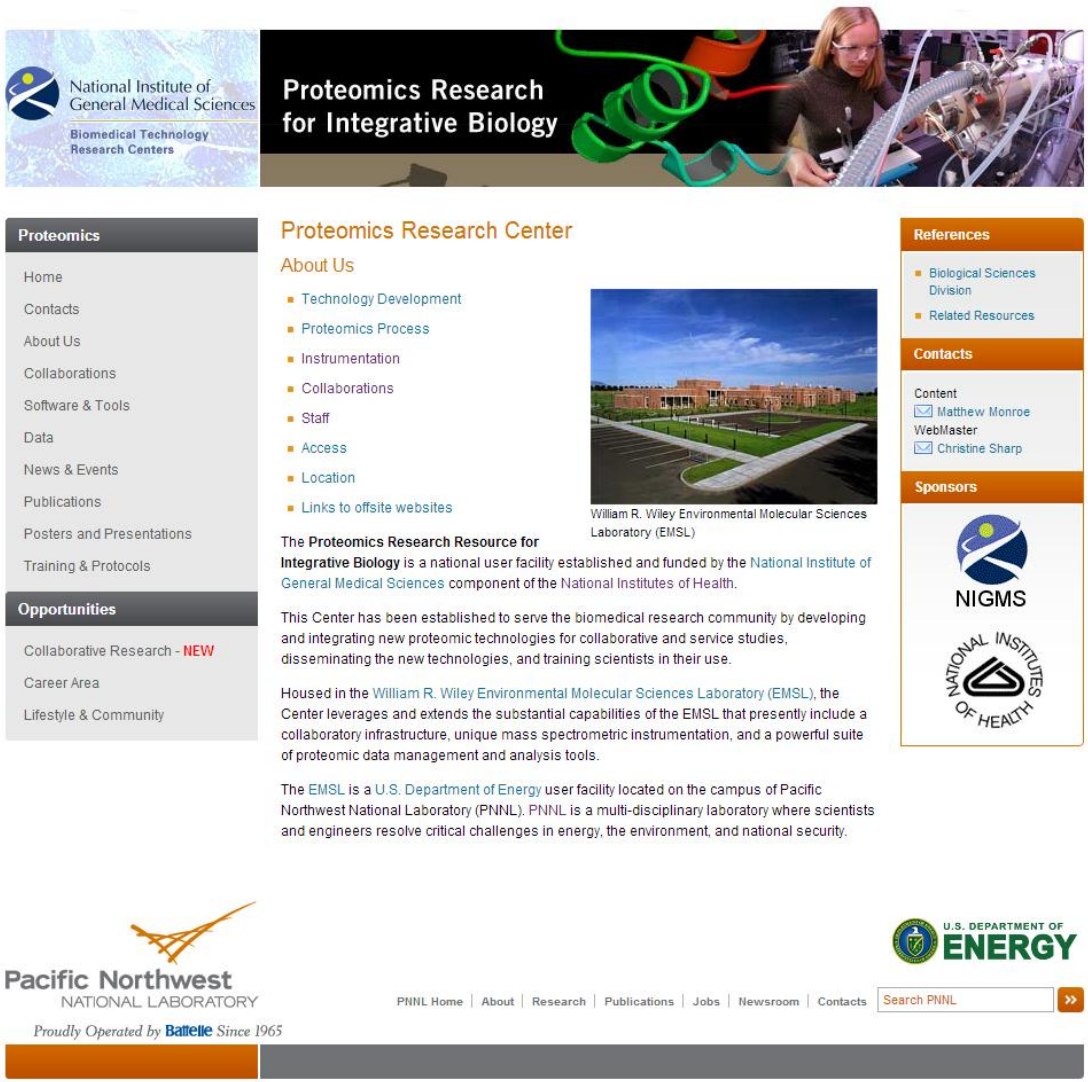
PNNL Proteomics



http://panomics.pnnl.gov/

- Quantitative Bottom-up LC-MS proteomics
  - Large scale (> 1,000 samples) studies
  - Post-translational modifications:
    - Phosphoproteomics: pS/T, pY
    - Glycosylation: N-GlcNAc, O-GlcNAc
    - Glycation: advanced glycation endproducts (AGE)
    - Oxidative damage: methionine oxidation, tyrosine nitration
- Top-down analysis of intact proteins
  - Protein isoforms
  - Combinations of post-translational modifications
  - Proteolytic fragments
- Protein turnover
- Activity-based proteomics
- Targeted SRM quantitation of low-abundant proteins

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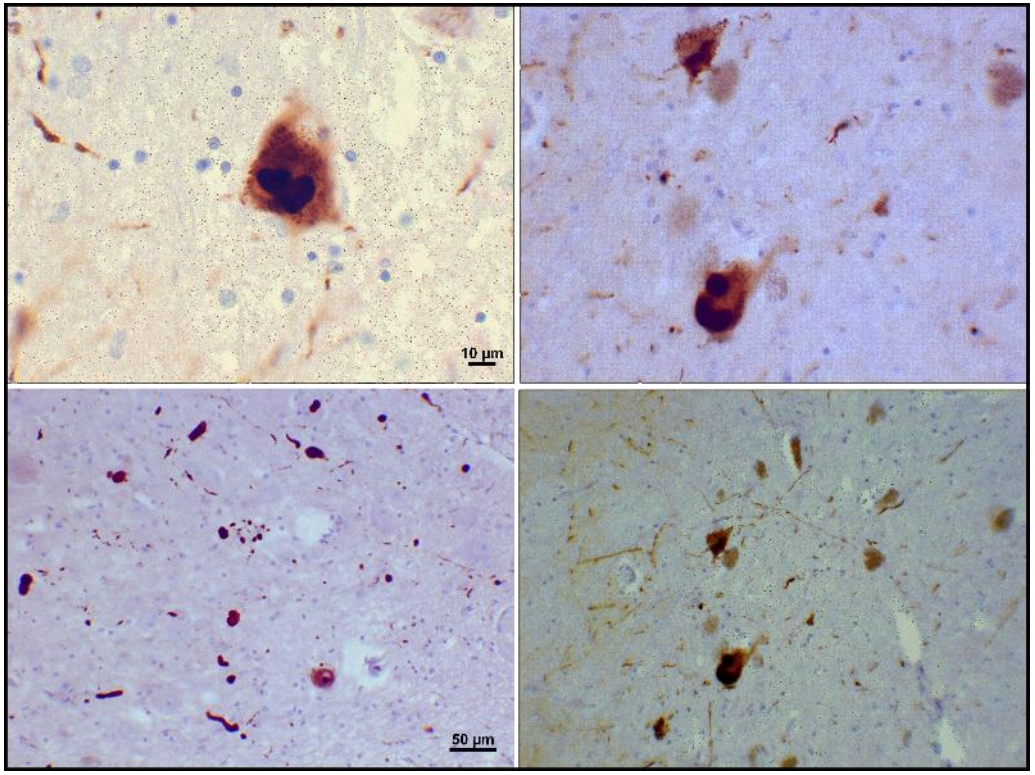
Lewy Bodies

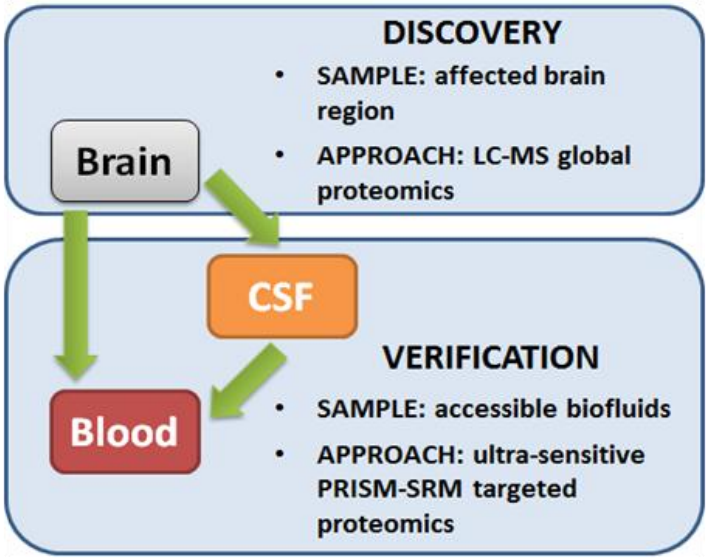


Photomicrographs of regions of substantia nigra in this Parkinson's patient show Lewy bodies and Lewy neurites in various magnifications. Top panels show a 60-times magnification of the alpha-synuclein intraneuronal inclusions aggregated to form Lewy bodies. The bottom panels are 20x magnification images that show strand-like Lewy neurites and rounded Lewy bodies of various sizes. Neuromelanin-laden cells of the substantia nigra are visible in the background.
http://en.wikipedia.org/wiki/Lewy_body

The Premises for Successful Development of Biofluid Biomarkers

- There are differences in abundances of proteins, their isoforms or post-translationally modified forms between LB-containing and LB-free brain tissue specimens.

- Those proteins/isoforms/modifications are:
  - Brain or even brain-region specific (thus avoiding dilution issue)
  - > 2-fold Increase in abundance in the case of pathologic condition (debatable)
  - Infiltrate the blood with reasonable efficiency (i.e. either highly abundant or effectively secreted)



Specific Aims

- **1. Identify proteins that correlate with the presence of the Lewy bodies in brain tissue.** We will perform statistical analysis of quantitative LC-MS proteomics data that represents brain tissue samples of 500 subjects from the Religious Orders Study (ROS) and Memory and Aging Project (MAP) cohorts. Proteins that exhibit significant increases in abundance associated with the presence of Lewy bodies and are expressed exclusively in the brain tissue will be considered as biomarker candidates for subsequent verification in the CSF and blood.
- **2. Verify the measured abundances of putative LB biomarkers in CSF and blood serum biofluids, correlate the results with brain tissue abundances within the same subjects, and develop a predictive model for LB presence.** We will measure extremely low levels of brain specific proteins in CSF and blood serum by taking advantage of a recently developed ultra-sensitive SRM approach. We will select proteins that show statistically significant correlations between the abundance profiles for brain tissue and the corresponding biofluid. Selected biomarkers will be utilized to develop a statistical model for predicting the LB load in the brain.
- **3. Validate the blood-based LB predictive model using an independent PD case/control cohort.** Protein biomarkers will be quantified in serum from 20 PD patients and matched controls available from the Osteoporotic Fractures in Men Study (MrOS) cohort. The predictions derived from the model will be matched against the clinical data to assess the agreement.
- **Statement of Impact.** The proposed project fundamentally aims to understand the relationships between protein abundance profiles in brain and biofluids such as CSF and blood. Reflection of the brain proteome in these biofluids would open a path for biomarker development for a number of neurodegenerative and other brain-related disorders. The successful outcome of the project will demonstrate the feasibility of following-up brain specific proteins in the CSF and especially in blood via ultra-sensitive SRM technology. A specific outcome of the project includes a statistical model for predicting LB load based on protein abundances in either blood serum or CSF, or both biofluids. We believe that the biofluid based LB assay will be a valuable addition to the toolbox for diagnostics and drug trials.

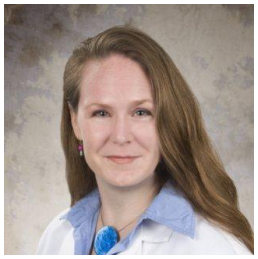
Ongoing Collaboration

David Bennett
Rush University

Director of Rush Alzheimer's Disease Center.
Longitudinal studies of age-onset brain disorders and cognitive impairment. ~3,000 participants. Post-mortem organ donation.

500 well-characterized frontal gyrus brain tissue samples

400+ frontal gyrus samples



Amanda Myers
University of Miami

Population genetics

Study design,
Data integration,
Network inference

Vlad Petyuk
Pacific Northwest National Lab

Proteomics

LC-MS analysis of 982 subjects

5R01AG034504
BRAINOME: Genome, transcriptome and proteome interaction in human cortex.

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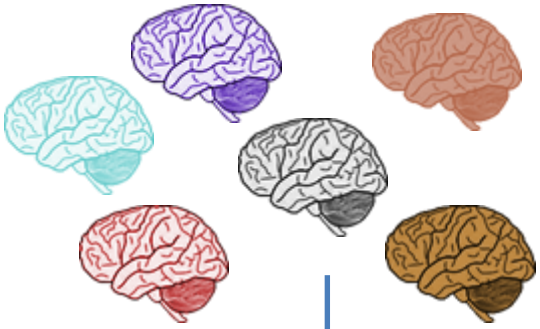
Experimental Design for Protein Quantitation Across 982 Brain Tissue Samples

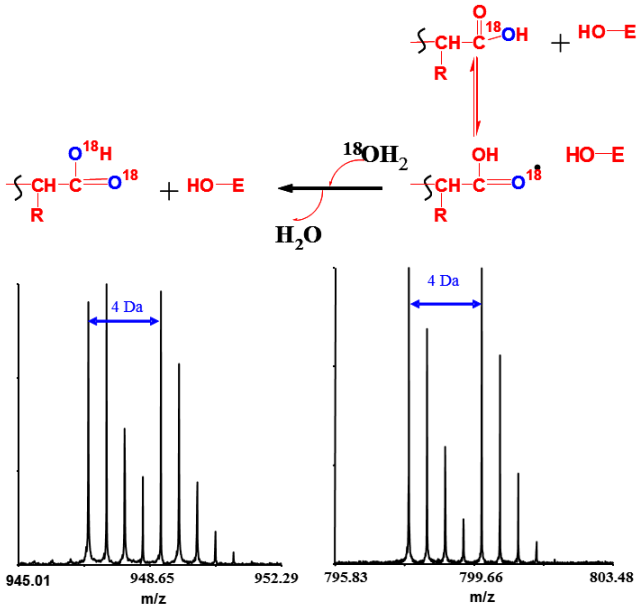


Pooled aliquot as a reference

Stable isotope labeling of the peptides with $2xO^{18}$

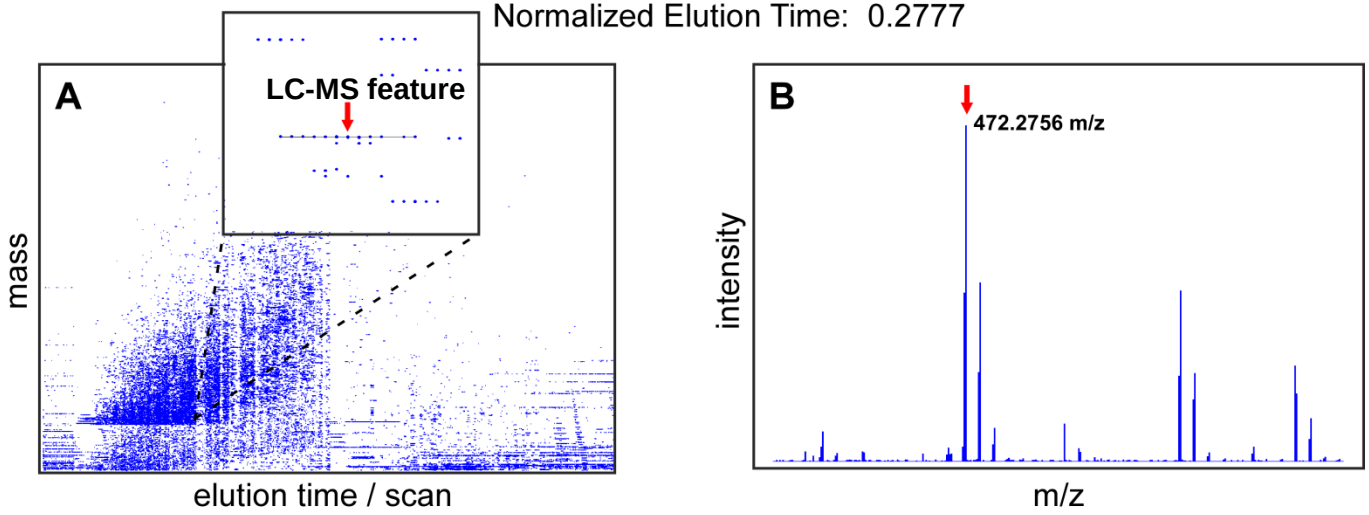
Spiking-in labeled reference into individual samples

LC-MS quantitation of peptides using O^{16}/O^{18} isotope ratios



Peptide Identification by Mass and Elution Time

Monoisotopic Mass: 942.5392 Da
Normalized Elution Time: 0.2777



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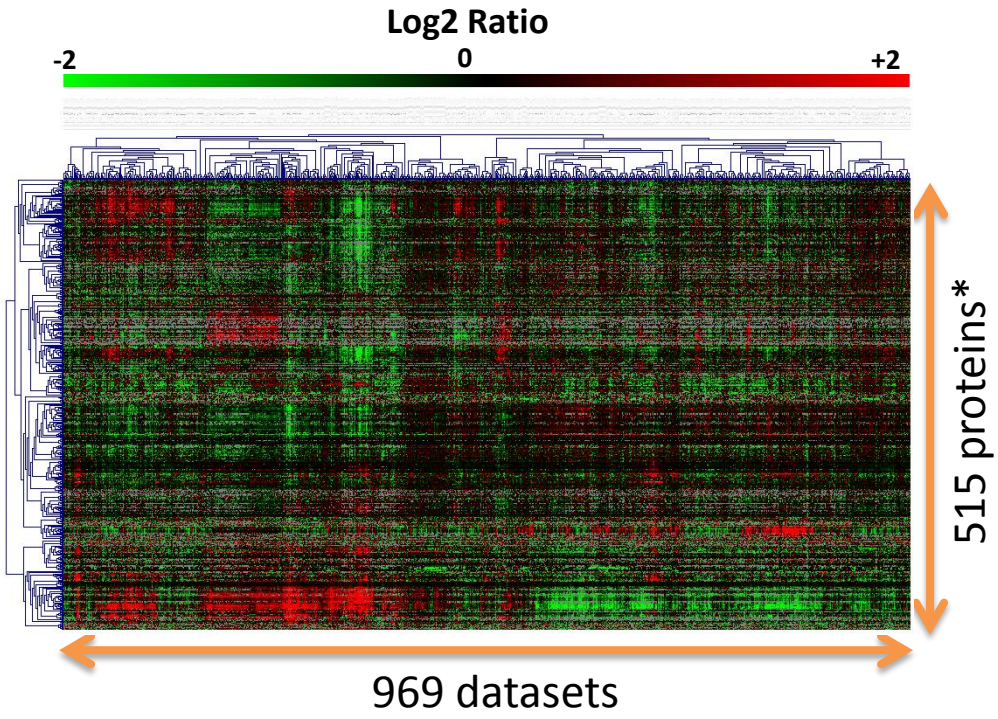
PEPTIDE	MASS	NET	ΔMASS (PPM)	ΔNET
ITPELLTR	941.5545	0.2923	-1045.8	***0.015***
SGKPAELLK	941.5545	0.2285	-1045.8	-0.049
VLQIVTNR	941.5658	0.2487	-1033.9	-0.029
RAVSQLIR	941.5770	0.2446	-1022.0	-0.033
FFEQMQN	942.3905	0.2857	-157.8	***0.008***
HELIEFR	942.4923	0.3087	-49.8	0.031
LTVPSADLK	942.5385	0.2659	***-0.7***	***-0.012***
TMYQVFR	943.4585	0.3033	974.4	0.026
HMLPSGFR	943.4698	0.2719	986.3	***-0.006***
LACGVIGIAQ	943.5160	0.3202	1035.3	0.042
AGLQFPVGR	943.5239	0.3150	1043.6	0.037
SATLFIHR	943.5239	0.2753	1043.6	***-0.002***
RGNVIMVR	943.5385	0.2349	1059.1	-0.043

Reasonable tolerances are:

- 2 ppm in mass measurement
- 0.02 deviation in elution time

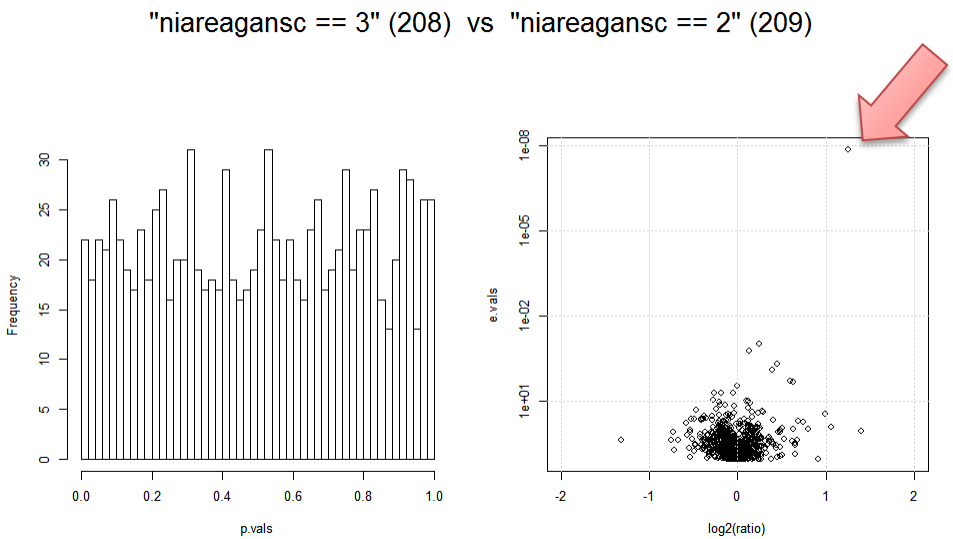
Acquired Proteomics Datasets



Sample Group	#
Pooled Controls	88
EURAD	93
EURCON	45
MAP	191
ROS	297
UKAD	65
UKCON	73
WGAAD	35
WGACON	82

*) Peptides for corresponding proteins required to be consistently present in at least 50% of pooled controls samples

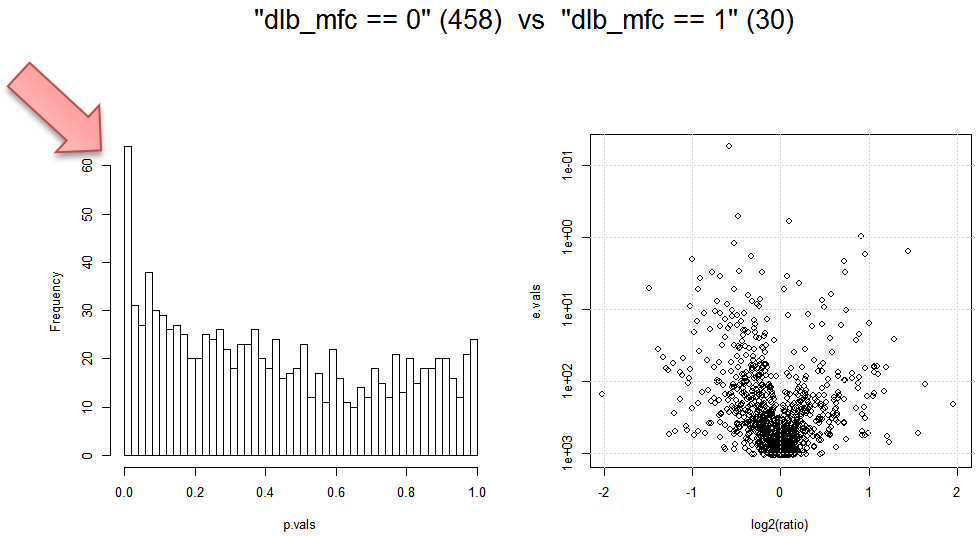
Differential Abundance Between Low and High NIA-Reagan Scores



UniProt	p.vals	FDR	e.vals	Log2 Ratio
A4_HUMAN	1.29E-11	1.37E-08	1.37E-08	1.257504
GFAP_HUMAN	8.92E-05	4.75E-02	9.50E-02	0.244635
KPCE_HUMAN	1.58E-04	5.60E-02	1.68E-01	0.135608
NDUS5_HUMAN	4.43E-04	1.18E-01	4.71E-01	0.444904
TLN2_HUMAN	7.45E-04	1.59E-01	7.93E-01	0.387727

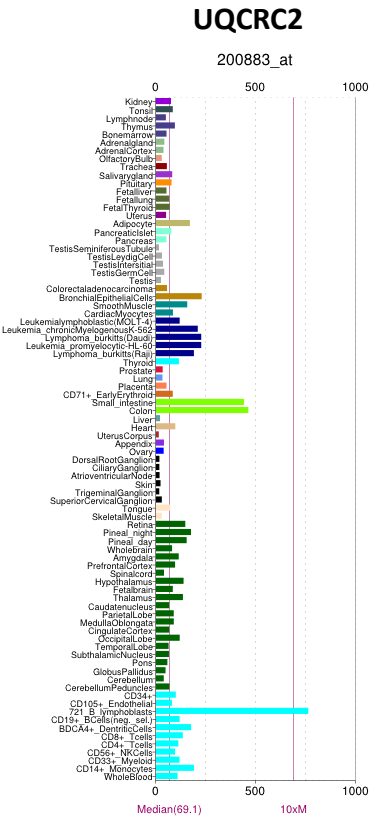
βA_{42} sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

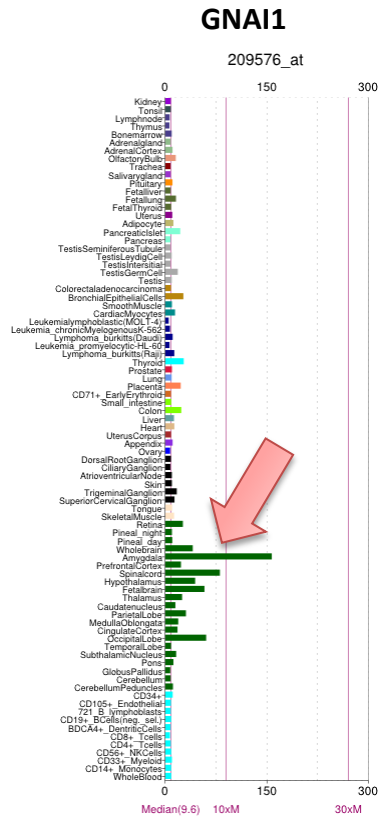
Differential Abundance Between Subject With and Without Lewy Bodies

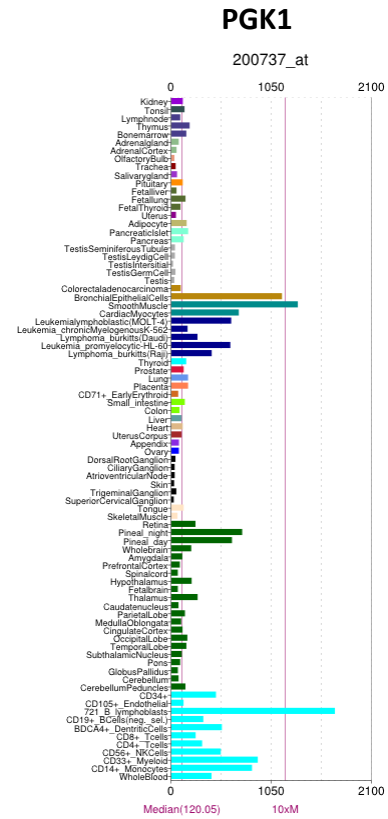


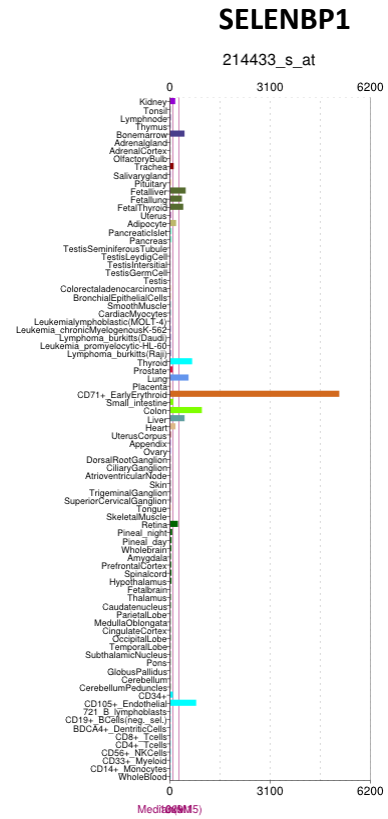
Gene	UniProt	p.vals	FDR	e.vals	Log2 Ratio	fold
UQCRC2	QCR2_HUMAN	5.32E-05	0.055733	0.055733	-0.58351	0.66
GNAI1	GNAI1_HUMAN	4.93E-04	0.258156	0.516311	-0.48109	0.71
PGK1	PGK1_HUMAN	5.86E-04	0.204765	0.614295	0.098997	1.07
SELENBP1	SBP1_HUMAN	9.35E-04	0.245086	0.980345	0.912769	1.87

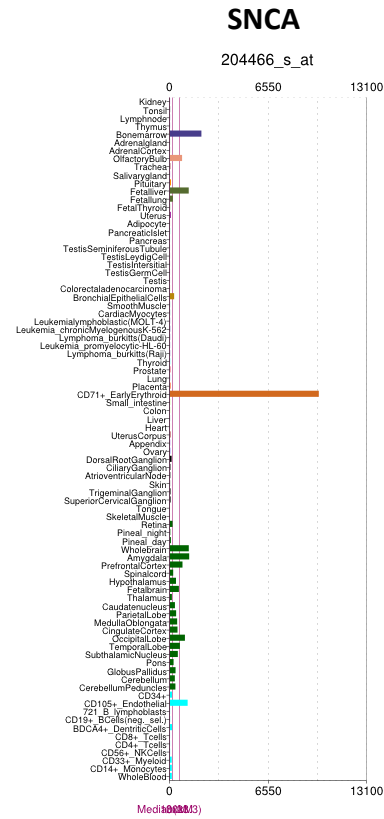
Tissue Specificity of Differentially Abundant Proteins











First month update: No good candidates, yet.

To Do:

1) **Statistical analysis accounting for confounding factors such as (sex, age, PMI and other pathologies).**
2) **Explore post-translational modifications**

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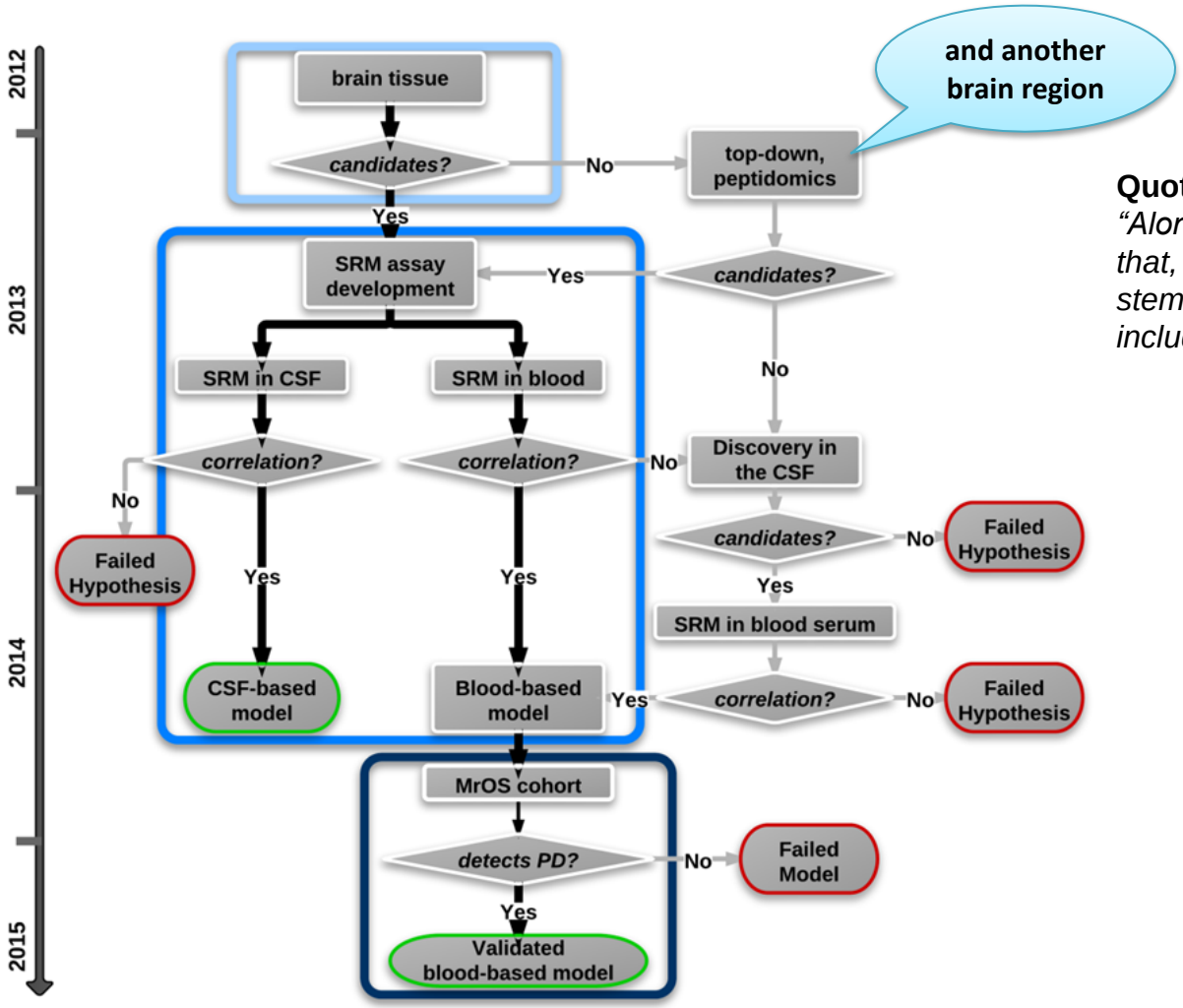
Main and Alternative Paths of the Project



Quote from the summary statement:
"Along these lines, the panel suggested that, in addition to cortical LB, LB in brain stem and substantia nigra should be included in the brain marker evaluation."

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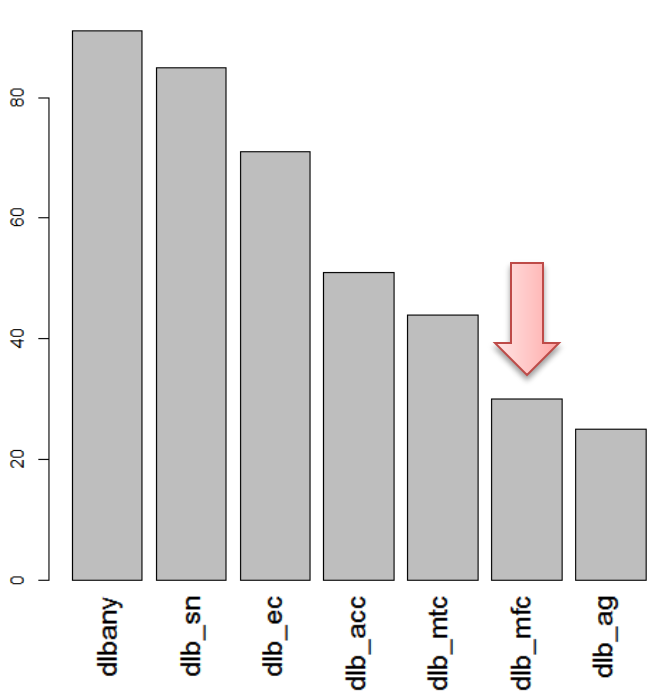
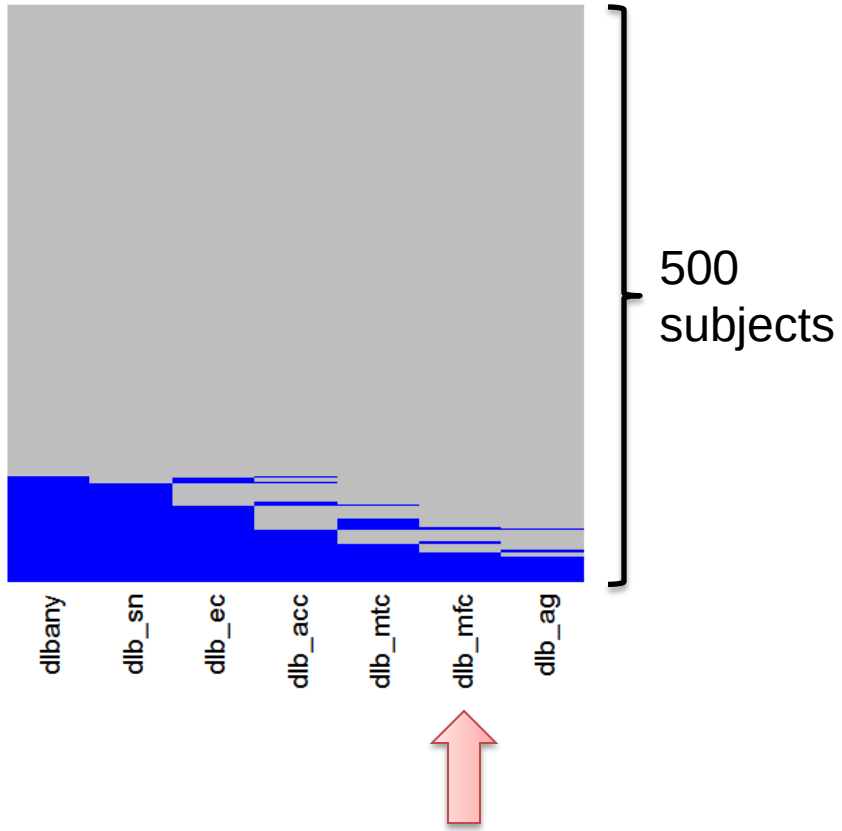
Distribution of LB Across Different Brain Regions





sn – substantia nigra
ec – entorhinal cortex
acc – anterior cingulate cortex
mtc – middle temporal gyrus
mfc – frontal gyrus
ag – angular gyrus

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Study Design (Aim 1)

Case – Control Design

~25 subjects without LB bodies in *Substantia nigra*

~25 subjects with LB bodies in *Substantia nigra*

Bottom-up & top-down LC-MS proteomics

List of proteins, isoforms or post-translational modifications increased (or even appeared) in LB+ group.

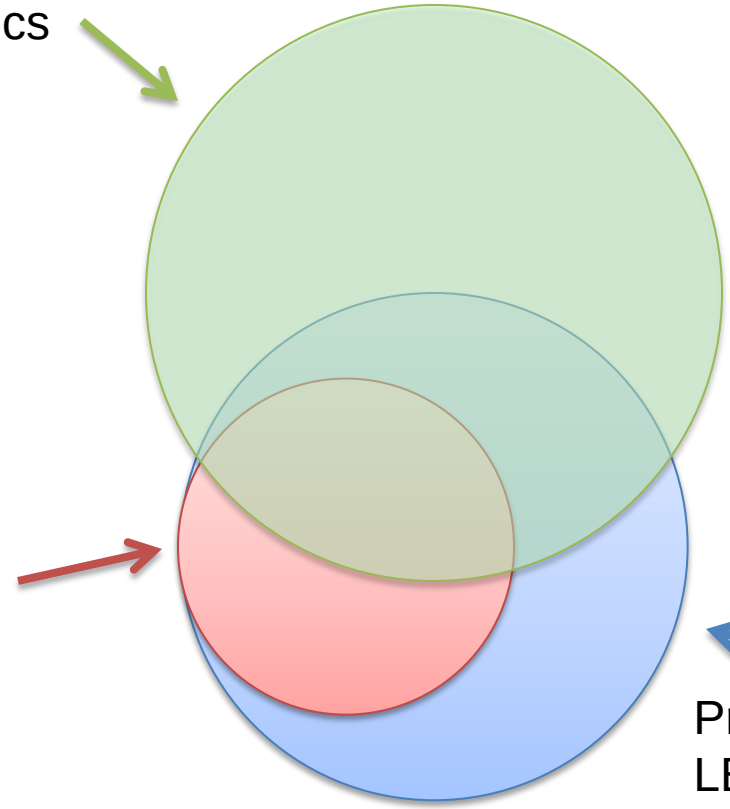
Next Qualification Step: Ensure SN (LB-containing SN) specificity of the discovered protein or proteoform candidates.

a) In the case of protein: Querying gene expression databases
b) In the case of proteoforms: Limited (top-down or targeted) LC-MS analysis of other abundant tissues

The Outcome of Top-Down Proteomics Experiments

Conceptual Venn Diagram

Proteoforms covered by top-down proteomics



Genes specific to substantia nigra

Proteoforms specific to LB+ substantia nigra

Ohrfelt A, Zetterberg H, Andersson K, Persson R, Secic D, Brinkmalm G, Wallin A, Mulugeta E, Francis PT, Vanmechelen E, Aarsland D, Ballard C, Blennow K, Westman-Brinkmalm A. Identification of novel α-synuclein isoforms in human brain tissue by using an online nanoLC-ESI-FTICR-MS method. Neurochem Res. 2011 Nov;36(11):2029-42. Epub 2011 Jun 16. PubMed PMID: 21674238;

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